AN EXPERIMENTAL STUDY OF POLYDISPERSE BACTERIAL AEROSOLS

Report II

Determination of the Chief Parameters of the Polydisperse System for a Calculation of the Survival of Pathogens

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Determination of the Chief Parameters of the Polydisperse System for a Calculation of the Survival of Pathogens

[Following is the translation of an article by V. P. Zhalko-Titarenko, Kiev Institute of Epidemiology and Microbiology, published in the Russian-language periodical Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii (Journal of Microbiology, Epidemiology and Immunobiology) No. 1, 1965, pages 123--129. It was submitted on 3 Feb 1964. Translation performed by Sp/7 Charles T. Ostertag Jr.]

In a previous report (ZhMEI, 1964, No 10) it was pointed out that in the study of bacterial aerosols we calculated the survival rate of bacteria (b_0) on the basis of three indices -- calculating concentration of the aerosol (C_c), concentration of viable microorganisms in the air (C_v) and the average number of microbes in the particles (h):

$$b_0 = \frac{C_v}{h \cdot C_c}.$$

While the determination of the countable concentration did not present a special difficulty thanks to the use of the VDK* ultramicroscope, the finding of the other values required special methodical processes. The most difficult was determining the average number of microbes in a particle. We determined this value in stained preparations of aerosol particles. When obtaining such preparations it is necessary to exclude the probability of secondary deformation and the breaking up of particles when they settled on the clean glass, ** therefore we were obliged to reject the inertial precipitators of the slotted type. Good results were obtained with the settling of the aerosol on glass in a field of high voltage current. The appropriate precipitator was designed by us based on an analogy with a common impactor

(figure 1). For a better settling of the particles the speed of the air current at the outflow of the feeding nozzle was reduced to 30 cm/sec. Thanks to this the weak inertial effect in the device was supplemented by the strong settling effect of the electric field with an intensity on the order of 15,000 V. The particles, without being deformed, became attached to a completely clean glass slide.

* [Probable meaning of VDK is Deryagin and Vlasenko's conveyer ultramicroscope.]

** The necessity of microscopic investigation of the structure of the particles excluded the use of the prevailing method of settling of particles on a layer of oil covering the glass.

Power supply for the precipitator was accomplished from a simple high voltage portable generator (designed by workers at the Voytsekhovskiy Institute). With the stated work regimen the effectiveness of settling for our models of aerosystems reached 0.99. This made it possible for us to consider that selectivity in the settling of various sized particles was not inherent to the device, and the average number of microbes in a particle was determined by the direct relation of the number of calculated microbes to the number of particles calculated in the same device.

There was a certain complexity in the staining of the particles and microbes. For studying freshly formed aerosols in saliva and in broth it was more convenient to study the microbial distribution in particles in models of aerosols, obtained from a preliminarily stained suspension. With this aim, directly before spraying we added a drop of Loffler blue per 1 ml of the initial suspension. The preparations were fixed in heat and microscoped. A liquid medium of particles formed a pale lilac background, on which stood out the dark bodies of the microbes with a normal circular form (figure 2).

Particles from aqueous suspensions and dried particles in saliva and broth are more favorably studied in unstained aerosols, since a drying liquid medium forms a very dark background and as a result it reduces the contrast of the image. Besides this, the stain apparently cements the adhering cells and doesn't permit them to go their separate ways when the preparation is treated with water. This also makes it difficult to calculate the individual cells. We stained the residue of the particles with stains which are usually used in bacteriological practice, but the period of staining was increased by many times (up to 45--60 min.). The lengthy exposure

not only promoted the thorough staining of the cell, but also broke up the compact structure of the dried particles, making it possible to easily distinguish the microbial cells.

The average number of microbes in a particle was calculated in a special series of model tests according to the formula:

$$\text{Average number of microbes in particle } h = \frac{\text{number of bacterial cells}}{\text{number of particles}}.$$

The results of the determinations were then transferred into relative values according to the formula: $i = \frac{h_n}{h_i}$.

With a concentration of the initial suspension equal to $14 \cdot 10^9$ cells in 1 ml, the largest drops of the precipitate of particles of a freshly formed aerosol contained up to 50--60 microbial cells. In a number of cases this made it difficult to get an accurate calculation of the number of microbes. Therefore the first measurements of the average number of microbes in a particle for such aerosystems must be extrapolated on the basis of test data, obtained in aerosols from suspensions with smaller cellular concentrations.

The results of a change in the average number of microbes in a particle during the course of a 2-hour exposure of aerosols in the chamber (figure 3) testified to the dependency of the value h on the concentration of the initial suspension. A noticeable difference was also noted in the change of the value h for particles from a suspension in saliva and in water with the same initial concentration. The lowering of the average number of cells in the particles with saliva took place more rapidly than in the aerosols from aqueous particles. Apparently this depended on the less volatility of the particles with the saliva; their weight proved to be greater than the weight of the corresponding aqueous drops, which led to the rapid precipitation of the large fractions of the aerosol into the residue.

We did not have the opportunity to measure the speed of evaporation of particles with a various composition of the liquid medium, though, as the recent works of Webb showed, such investigations have very great significance when studying the mechanism of necrosis of the aerial microflora. We obtained certain rough data as a result of analyzing microphotograms. While the particles on the aqueous base literally from the first test (1½ minutes after the formation of the aerosystem) had all the features of drying up, the drops from the saliva for around an hour retained remnants of moisture which were visible under a microscope, and dried particles appeared in a significant

quantity only at the end of the stated period (figures 4 and 5). This circumstance probably exerts an influence on the dynamics of necrosis of the microflora, which will be discussed in more detail in the next report.

The third parameter -- concentration of viable microbes in the aerosol -- requires the most accurate determination. Unfortunately many modern bacteria traps possess a low effectiveness or are distinguished by a considerable selectivity in catching particles of a various size. Therefore we were compelled to stay with the method of capturing particles with soluble granular aerofilters made from sodium alginate (Vershigora), since this method made it possible to increase the effectiveness of filtration up to the necessary extent by means of increasing the column of powder through which the aerosol passes. A deficiency of the method is that with an increase in effectiveness the resistance of the aerofilter increases, however, this can be tolerated in research work. An important advantage of the method is displayed during an investigation of polydisperse systems: Dissolving of the alginate granules leads to a sufficiently complete breakup of the particles, as a result of which a suspension from isolated cells is formed anew.

When seeding such material more reliable results are obtained than with other methods of trapping where an incomplete disintegration of the cellular aggregates of the particles is possible. In analyzing the residue of microbes, obtained by centrifuging a solution of alginate which had been preliminarily exposed in an aerosol of the diphtheria bacillus, we were satisfied with a sufficiently thorough breakup of the particles. The diffuse, nonaggregate distribution of the microbes indicated the full-value disintegration of the particles (figures 2 and 6).

For preparation of the filters we used screened, minute powder with granules less than 100 microns. The alginate was poured in a layer of 0.3 cm into a little tube with the ends constricted with a little wad of cotton. When taking a sample the alginate together with the cotton caused a rarefaction equal to 0.4 kg/ml^2 and the air was filtered with a speed capacity of 50 ml/sec and a linear speed of 700 cm/sec.

At such a work regimen the effectiveness of the aerofilter approached 1. This could be determined by measuring the calculation concentration of the aerosol before and after it is passed through the aerofilter. With such a method of checking effectiveness the result obtained was equal to 0.988 (98.8%) of capturing). The aerofilters were also checked by the bacteriological method. Two small tubes with alginate were connected consecutively and through them a bacterial

aerosol with diphtheria bacilli was drawn. Seedings from the tubes showed that in the second (along the path of the aerosol) tube diphtheria bacilli were absent, while from the first one hundreds of microbial cells were seeded out (table 1). Control seedings from cotton plugs onto which alginate was poured, were practically sterile. Therefore the effectiveness based on the passage of bacterial particles turned out to be equal to 1 (100% capture). The small difference in both determinations of effectiveness (0.988--1) can apparently be regarded as due to presence of a small quantity of very fine microbe less particles, which cannot be released even with a considerable saturation of the initial suspension with microbial cells. Probably these particles are very small and pass through the filter.

Since sodium alginate is used very little in bacteriological practice, it was necessary to make a detailed check of its harmlessness for the growth and multiplication of a culture of diphtheria bacilli. With this aim, following the passage of the aerosol we maintained the filters without dissolving them for a period of an hour, and over the same time period we maintained the dissolved filters. After the expiration of the stated period the undissolved alginate was dissolved and we made seedings from it. At the same time we made seedings from the alginate which was dissolved immediately after the sample was taken. The selected time interval (one hour) exceeded by three times the time which aerofilters may be left without use.

The tests showed that the number of microorganisms detected remains without essential changes throughout the entire hour of exposure (table 2).

Thus the method of capturing the suspended phase of an aerosol with the help of granular filters turned out to be suitable for studying polydisperse systems within the limits of our methods of treatment with this material and those problems which stood before us.

Both of the described methods -- capturing the particles with the help of sodium alginate and calculating the average number of microbes in a particle -- are conditioned by the characteristics of polydisperse systems. Since polydispersity is the most common and typical property of aerosols (because even the so called nondisperse systems all told are only a laboratory model of an aerosol with a relatively small interval of particle sizes), then the proposed methods are necessary on the whole for the entire methodology of studying bioaerosystems. The methodical solving of the problems proposed by us is not unique, and we feel that further searches for new solutions are absolutely necessary, since the methods described here are calculated on the basis of research practice under laboratory conditions.

Conclusions

1. A study of the nature of a bacterial aerosol requires a determination of the average content of microbes in the particle.

2. An electrostatic precipitator is proposed for the preparation of preparations of particles with the aim of investigating them microscopically.

3. For obtaining stained preparations, two methods are used -- staining of the cells in the initial suspension prior to spraying in the chamber (for studying freshly formed aerosols), and staining the particles on glass by the lengthy exposure of the pigment (for aerosols with dried particles).

4. The sedimentation phenomena in a polydisperse aerosol proceed with a primary precipitation of large undried particles, that is why the average number of cells in a particle decreases in aerosols in saliva more rapidly than in aqueous aerosols.

5. Drying particles are predominant in aqueous aerosols immediately after their formation, and in aerosols in saliva -- only in an hour after the spraying of the initial suspension.

6. Satisfactory results in determining the survival rate of microbes in a polydisperse aerosol are guaranteed by a soluble granular aerofilter made of sodium alginate, since it makes it possible to effectively capture bacterial aerosols and following dissolving leads to the breaking up of the cellular complexes of the particles. Alginate turned out to be a harmless substance for the diphtheria bacillus.

Literature

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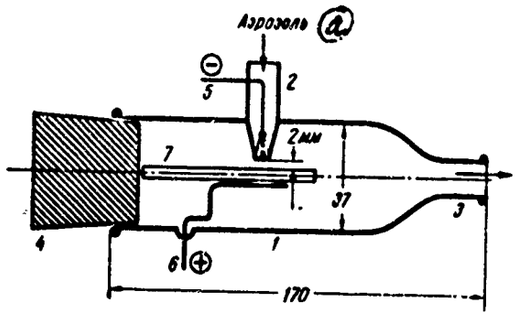

Figure 1. Precipitation cuvette. a - aerosol; 1 - body of cuvette; 2 - feeding tube; 3 - discharge tube; 4 - stopper; 5 - negative brush electrode; 6 - positive electrode; 7 - glass slide.

Figure 2. Particles of a freshly formed aerosol of diphtheria bacilli in salive (stained suspension).

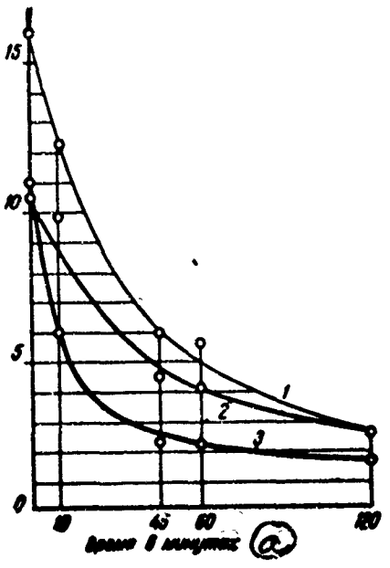

Figure 3. Average content of microbes in a particle depending on the concentration of the initial suspension and the time of exposure in the chamber. a - time in minutes; 1 - 14 • 10^9 cells in 1 ml of an aqueous suspension; 2 - 10 • 10^9 cells in 1 ml of an aqueous suspension; 3 - 10 • 10^9 cells in 1 ml of a suspension in saliva.

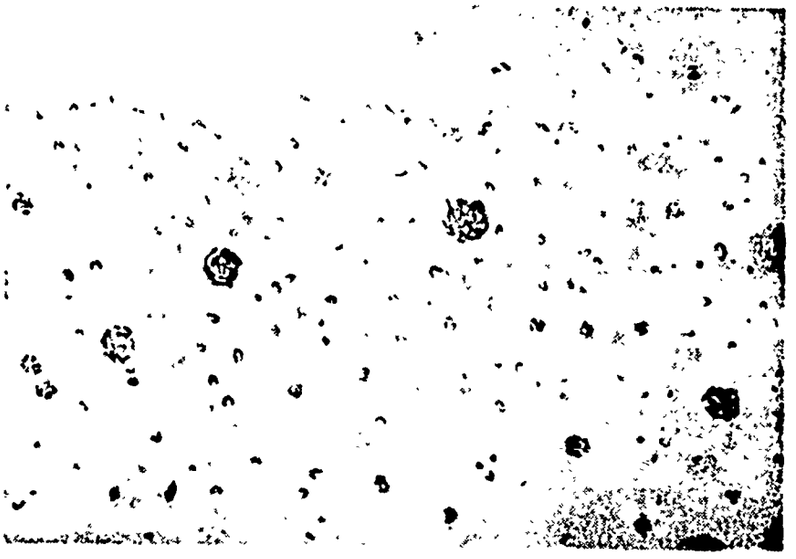

Figure 4. Particles of a freshly formed aerosol of diphtheria bacilli in water (staining on the glass).

Figure 5. Particles of an aerosol of diphtheria bacilli in saliva after 60 minutes following spraying. Arrows indicate dried particles (stained suspension).

Figure 6. Breaking up of the cellular complexes of particles of an aerosol of diphtheria bacilli following precipitation and dissolving of sodium alginate.

Table 1

Testing of aerofilters with sodium alginate for the passage of a suspended phase of an aerosol of diphtheria bacilli.

No.	Number of colonies which grew in each of five dishes out of the aerofilter being tested	Control of the passage of particles (seeding out of the 2nd aerofilter in five dishes)
1	403, 366, 362, 371, 269	0, 0, 0, 0, 0
2	551, 402, 433, 474, 465	0, 0, 0, 0, 0
3	404, 267, 498, 415, 490	0, 0, 0, 0, 0
4	314, 284, 229, 296, 426	0, 0, 0, 0, 0
5	403, 426, 387, 430, 446	0, 0, 0, 0, 0
6	404, 463, 300, 328, 328	0, 0, 0, 0, 0
7	342, 341, 274, 280, 287	0, 0, 0, 0, 0
8	359, 284, 296, --- ---	0, 0, 0, 0, 0
9	320, 292, 303, 415, 316	0, 0, 0, 0, 0
10	298, 393, 359, 366, 342	0, 0, 0, 0, 0

Table 2

Survival rate of diphtheria bacilli in a solution and in a powder of sodium alginate.

No. of test	No. of tube with alginate	Number of colonies following seeding from the tubes immediately after the sample was taken (in 10 dishes)	Number of colonies following seeding after an hour
1	1	4310	4910
	2	4770	3820
	3 (solution)	5000	4100
	4	3980	4040
	5	4640	4290
	6	4200	4690
	7		5230
	8		4910
	9 (left for an hour in an		4690
	10 undissolved form)		3810
	11		3970
	12		4000
2	1	180	190
	2 (solution)	172	240
	3	210	240
	4 (left for an hour in an		275
	5 undissolved form)		220
	6		167
3	1	4030	3940
	2 (solution)	3660	4640
	3	3510	4150
	4	4020	4900
	5	4040	4980
	6		4080
	7 (left for an hour in an		4260
	8 undisolved form)		4444
	9		4470
	10		5100